Relation of coronary heart disease and apolipoprotein E phenotype in patients with non-insulin dependent diabetes

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Abstract

Objectives—To examine the relation between coronary heart disease and the apolipoprotein E phenotypes in patients with non-insulin dependent diabetes mellitus.

Design—Cross sectional study.

Setting—District around Kuopio University Central Hospital, East Finland.

Subjects—138 men with non-insulin dependent diabetes and 64 men without diabetes as controls.

Main outcome measure—Apolipoprotein E phenotype, electrocardiographic abnormalities, other signs of coronary heart disease.

Results—The prevalences of definite myocardial infarction and ischaemic electrocardiographic changes were highest in the diabetic men with the phenotypes E4/4 or E4/3 (25% (95% confidence interval 18% to 32%) and 50% (42% to 58%) respectively), although the difference between the phenotype groups was not significant. The prevalence of angina pectoris was 69% (61% to 77%) in men with the phenotypes E4/4 or E4/3 ($p=0.005$ compared with other phenotypes), 41% (33% to 49%) in men with phenotype E3/3, and 47% (39% to 55%) in those with phenotypes E2/2 or E2/3. Similarly, the simultaneous presence of angina pectoris and ischaemic electrocardiographic changes was highest in the diabetic men with the phenotypes E4/4 or E4/3 (42% v 22% in those with E3/3 and 29% in those with E2/2, E2/3; $p=0.038$). Overall, the prevalence of any evidence of coronary heart disease among the diabetic subjects with the phenotypes E4/4 or E4/3 was 81% ($p=0.011$ compared with other phenotypes), 58% in those with phenotype E3/3, and 53% in those with phenotypes E2/2 or E3/3.

Conclusion—Apolipoprotein E phenotypes E4/4 and E4/3 modulate the risk of coronary heart disease in men with non-insulin dependent diabetes.

Introduction

Lipoproteins are important determinants of atherosclerotic vascular disease in man. The serum concentrations and metabolism of lipoproteins are largely modulated by apolipoproteins and it has therefore been hypothesised that genetic variations in apolipoproteins are associated with the variation in the susceptibility to coronary heart disease.¹ Polymorphism of apolipoprotein E has recently received an increasing attention. Three common alleles, e2, e3, and e4 determine the six apolipoprotein E phenotypes E2/2, E2/3, E2/4, E3/3, E4/3, and E4/4. Low density lipoprotein metabolism is associated with the apolipoprotein E polymorphism such that subjects with the phenotypes E4/3 and E4/4 have higher concentrations and subjects with the e2 allele lower plasma concentrations of total and low density lipoprotein cholesterol than do subjects with the commonest apolipoprotein E phenotype, E3/3.^{2,5}

Diabetic patients, particularly those with non-insulin dependent diabetes, have an increased risk of all manifestations of atherosclerotic vascular disease.⁶ Although non-insulin dependent diabetes has adverse effects on serum lipoproteins and is associated with an increased incidence of hypertension and obesity, the strength of the relation between total cholesterol concentration, blood pressure, and smoking and the risk of coronary heart disease seems to be about the same in people with and without diabetes.⁶ Indeed, the major proportion of the excess of coronary heart disease in non-insulin dependent diabetic patients still remains unexplained.

The Finnish population has an extremely high prevalence of coronary heart disease, and Finland also ranks among those countries with the highest cholesterol concentrations in the world.⁷ Among the Finns men with non-insulin dependent diabetes from East Finland are at a particularly high risk of atherosclerosis; manifestations of coronary heart disease occur two to four times more often among them than in men without diabetes.⁸ Since classic risk factors do not seem to explain this excess risk⁹ we studied the relation between coronary heart disease and apolipoprotein E phenotypes in men with non-insulin dependent diabetes from East Finland to elucidate the role of the phenotype pattern in modulating the risk of coronary heart disease.

Subjects and methods

All diabetic patients in Finland needing drugs for diabetes are provided with them free of charge according to the Sickness Insurance Act. The Social Insurance Institution maintains a central register of all the diabetic subjects receiving drug reimbursement. From this register all diabetic subjects aged 45-64 years were identified from the population living in the district of Kuopio University Central Hospital (East Finland). The subjects were originally identified for a study of the prevalence of atherosclerotic vascular disease and its risk factors in subjects with and without diabetes.⁸ For the present study we randomly selected 138 of the 253 men with non-insulin dependent diabetes. All diabetic men included in the final study population fulfilled the World Health Organisation criteria for diabetes mellitus.⁹

We randomly selected 64 men without diabetes, aged 45-64 years, from the population register for the district to serve as controls. The controls were thus completely representative of the non-diabetic population of the study area. Diabetes was excluded in the controls on the basis of measurements of fasting plasma glucose concentration. None of the control or diabetic men were receiving hypolipidaemic drugs and none had a significant impairment in renal function as assessed by serum creatinine concentration. The participation rate in the original study from which

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these men were drawn was 79% in the control group and 81% in the diabetic group.⁸

Body mass index was calculated according to the formula weight(kg)/height(m)². Exercise level was divided into two categories: (a) no or slight physical exercise during leisure time or work and (b) regular physical exercise during leisure time (for example, walking, bicycling, jogging, or swimming for at least 30 minutes at least twice a week) or heavy physical activity at work (for example, heavy industrial work, farming, lumberjacking), or both. Smoking was defined on the basis of current cigarette smoking. Hypertension was defined as the use of antihypertensive drugs or actual systolic blood pressure >160 mm Hg or diastolic blood pressure >95 mm Hg. Angina pectoris was evaluated by the Rose cardiovascular questionnaire.¹⁰

The medical records of all those subjects who reported having been admitted to any hospital because of chest pain were carefully reviewed. We used the WHO criteria for definite myocardial infarction, based on the chest pain symptoms, electrocardiographic changes, and enzyme determinations, to diagnose previous myocardial infarction.¹¹ A conventional electrocardiogram was recorded from each subject after a 12 hour fast; Abnormalities were classified according to the Minnesota code.¹⁰ The statistical analyses concerning the association of apolipoprotein E phenotypes with coronary heart disease were carried out in the following categories: definite myocardial infarction (definite myocardial infarction verified at hospital or Q/QS change on electrocardiography, Minnesota codes 1.1-1.2); ischaemic electrocardiographic changes (ischaemic ST/T changes or left bundle branch block, Minnesota codes 1.1-1.3, 4.1-4.3, 5.1-5.3, 7.1); angina pectoris; and any evidence of coronary heart disease (definite myocardial infarction or ischaemic electrocardiographic changes or angina pectoris).

LABORATORY TESTS

Serum lipid and lipoprotein concentrations were determined from fresh serum samples drawn after a 12 hour overnight fast. Lipoprotein fractionation was performed by ultracentrifugation and selective precipitation with a modification¹² to the method originally described by Havel *et al*¹³ as previously reported.^{14,15} Cholesterol and triglycerides concentrations in whole serum and in lipoprotein fractions were assayed by automated enzymatic methods (Boehringer-Mannheim, West Germany).¹⁶ Serum apolipoprotein AI concentration was determined by a commercial immunochemical method that was based on measuring immunoprecipitation at 340 nm (Orion Diagnostica, Finland).¹⁷ Serum apolipoprotein B concentration was determined by electroimmunoassay.¹⁸ The apolipoprotein E phenotype was determined from the whole plasma after delipidation by isoelectric focusing and immunoblotting techniques.^{19,20}

The concentration of glycated haemoglobin (normal range 5.5-8.5%; coefficient of variation 6%) was determined by commercial column chromatography (Quick-Sep fast haemoglobin test system, Isolab Akron, Ohio, United States) after incubation of blood in 0.9% saline for 12 hours. Plasma glucose concentration was measured by the glucose dehydrogenase method (Merck Diagnostica, West Germany). Patients' endogenous insulin secretion capacity was assessed after eating by measuring plasma C peptide concentration after stimulation with 1 mg of intravenous glucagon.²¹ Plasma C peptide was assessed by radioimmunoassay (antiserum M 1230, Novo Industri, Copenhagen, Denmark).²² The diagnosis of non-insulin dependent diabetes was based on criteria

suggested by the WHO.⁹ In insulin treated patients the C peptide response to intravenous glucagon was used to classify different types of diabetes. Patients with a C peptide concentration over 0.2 nmol/l six minutes after 1 mg of glucagon with no history of ketoacidosis were classified as having non-insulin dependent diabetes. Patients with no or low C peptide response (<0.20 nmol/l) were diagnosed as having insulin dependent diabetes mellitus²³ and excluded from the study.

STATISTICAL METHODS

Results for continuous variables are given as mean (SD) values. Comparisons between more than two groups were done by the analysis of variance. Comparisons between two groups were performed by the χ^2 test, Fisher's exact test, or Student's *t* test for independent samples. Logarithmic transformation of total and very low density lipoprotein triglyceride concentrations were used in all statistical analyses including these variables.

Results

Table I lists the characteristics of the study groups. Diabetic subjects were heavier, had more hypertension, and were less physically active than the control subjects. Table II gives the frequency of the various apolipoprotein E phenotypes and the gene frequencies. Although the phenotype E4/3 was somewhat more common among men with non-insulin dependent diabetes, no significant differences between the diabetic and control groups were observed. Correspondingly, gene frequencies of the alleles e2, e3, and e4 did not differ between the two groups.

Serum low density lipoprotein cholesterol concentration was higher in subjects with the phenotypes E3/3, E4/4, or E4/3 compared with that in subjects with the phenotypes E2/2 or E2/3. Otherwise serum lipid and lipoprotein concentrations did not differ among the apolipoprotein E phenotypes (table III). Serum total cholesterol and apolipoprotein B concentrations tended to be higher in subjects with the phenotypes E4/4 or E4/3 than in the other groups, but the difference did not reach significance. With respect to clinical characteristics and metabolic control of diabetes the apolipoprotein E phenotype groups did not differ significantly from each other (table IV). However, the

TABLE I—Characteristics of control subjects and subjects with non-insulin dependent diabetes

	Controls	Diabetic men	p Value
No of Men	64	138	
Mean (SD) age (years)	54 (1)	56 (1)	NS
Mean (SD) body mass index (kg/m ²)	25.5 (0.3)	27.7 (0.3)	<0.001
Mean (SD) fasting blood glucose (mmol/l)	5.4 (0.1)	10.8 (0.3)	<0.001
Mean (SD) glycated haemoglobin (%)	7.7 (0.2)	9.6 (0.2)	<0.001
No (%) of smokers	18 (29)	37 (27)	NS
No (%) with hypertension	13 (20)	85 (62)	<0.01
No (%) physically active	45 (70)	68 (49)	<0.05

TABLE II—Apolipoprotein E phenotypes and gene frequencies in control and diabetic men

	Control (n=64)	Non-insulin dependent diabetes (n=138)
Phenotype (No (%)):		
E2/2	2 (3)	2 (1)
E2/3	4 (6)	15 (11)
E3/3	46 (72)	85 (62)
E4/3	10 (16)	32 (23)
E4/4	2 (3)	4 (3)
Gene frequency:		
e2	0.063	0.069
e3	0.828	0.786
e4	0.109	0.145

TABLE III—Mean (SD)* serum lipid, lipoprotein, and apolipoprotein concentrations in men with non-insulin dependent diabetes by apolipoprotein E phenotype

	E2/2, E2/3 (n=17)	E 3/3 (n=85)	E4/3, E4/4 (n=36)	Analysis of variance (p value)
Cholesterol (mmol/l):				
Total	6.33 (1.94)	6.69 (1.27)	6.78 (1.53)	NS
High density lipoprotein	1.11 (0.27)	1.16 (0.31)	1.08 (0.29)	NS
Low density lipoprotein	3.59 (0.94)	4.26 (0.94)†	4.15 (1.06)†	0.038
Very low density lipoprotein	1.63 (1.42)	1.28 (1.01)	1.55 (1.30)	NS
Triglycerides (mmol/l):				
Total	2.07 (1.31 to 2.83)	2.07 (1.78 to 2.36)	2.10 (1.63 to 2.57)	NS
High density lipoprotein	0.13 (0.08)	0.15 (0.08)	0.13 (0.07)	NS
Low density lipoprotein	0.35 (0.16)	0.40 (0.22)	0.37 (0.21)	NS
Very low density lipoprotein	1.57 (0.71 to 2.51)	1.51 (0.85 to 1.85)	1.60 (1.08 to 2.12)	NS
Apolipoprotein (g/l):				
AI	0.76 (0.10)	0.76 (0.13)	0.75 (0.15)	NS
B	0.90 (0.30)	1.01 (0.36)	1.06 (0.34)	NS

*Geometric means (95% confidence intervals) are presented for total and very low density lipoprotein triglycerides.
†p<0.05 (E4/3, E4/4, v E2/2, E2/3 or E3/3 v E2/2, E2/3).

TABLE IV—Clinical characteristics and metabolic control for men with non-insulin dependent diabetes by apolipoprotein E phenotype

	E2/2, E2/3 (n=17)	E3/3 (n=85)	E4/3, E4/4 (n=36)	Analysis of variance (p value)
Mean (SD) age (years)	55 (1)	56 (1)	56 (1)	NS
Mean (SD) body mass index (kg/m ²)	28.1 (0.9)	27.7 (0.4)	28.1 (0.7)	NS
Mean (SD) fasting blood glucose (mmol/l)	11.3 (1.0)	10.7 (0.4)	10.3 (0.5)	NS
Mean (SD) glycated haemoglobin (%)	9.2 (0.5)	9.6 (0.2)	9.5 (0.3)	NS
No (%) of current smokers	3 (18)	25 (29)	8 (22)	NS
No (%) with hypertension	10 (59)	51 (60)	25 (69)	NS
No (%) physically active	13 (76)	41 (48)	18 (50)	NS

actual percentages of current smokers and physically active subjects were quite different among the groups.

Table V shows the prevalence of coronary heart disease by different criteria according to the apolipoprotein E phenotypes in men with non-insulin dependent diabetes. The prevalences of coronary heart

TABLE V—Prevalence of coronary heart disease by different criteria according to apolipoprotein E phenotype in non-insulin dependent diabetic men

	No of men	Prevalence (%)	95% Confidence interval (%)
Mycardial infarction:			
E2/2, E2/3	2	12	7 to 17
E3/3	15	18	12 to 24
E4/4, E4/3	9	25	18 to 32
Ischaemic electrocardiographic changes:			
E2/2, E2/3	6	35	27 to 43
E3/3	33	39	31 to 47
E4/4, E4/3	18	50	42 to 58
Angina pectoris:			
E2/2, E2/3	8	47	39 to 55
E3/3	35	41	33 to 49
E4/4, E4/3	25	69	62 to 75
Any evidence of coronary heart disease:			
E2/2, E2/3	9	53	51 to 71
E3/3	49	58	50 to 66
E4/4, E4/3	29	81	75 to 87

disease in control subjects were too low to allow statistical analysis. The prevalences of definite myocardial infarction and ischaemic electrocardiographic changes were highest in the subjects with the phenotypes E4/4 or E4/3 (25% and 50%, respectively), although the difference between the phenotype groups was not significant. Angina pectoris was found in 69% (25) of subjects with the phenotypes E4/4 or E4/3 (p=0.005 compared with other phenotypes) compared with 41% (35) of subjects with the phenotype E3/3 and 47% (six) of those with phenotypes E2/2 or E2/3. Similarly, the prevalence of simultaneous presence of angina pectoris and ischaemic electrocardiographic changes was higher (42%) in subjects with phenotypes E4/4 or E4/3 than in those with other phenotypes (E3/3 22%; E2/2, E2/3 29%; p=0.038). Overall, the prevalence of any evidence of coronary heart disease among the subjects with the phenotypes E4/4 or E4/3

was 81% (p=0.011 compared with other phenotypes), 58% in subjects with the phenotype E3/3, and 53% in subjects with phenotypes E2/2 or E2/3.

Discussion

The apolipoprotein E phenotypes E4/4 and E4/3 were strongly associated with coronary heart disease in non-insulin dependent diabetic men. This is a new finding which could explain, at least in part, the increased risk of coronary heart disease in patients with non-insulin dependent diabetes.

The evidence that the apolipoprotein E polymorphism modulates susceptibility to atherosclerotic vascular disease in people without diabetes is controversial. Some studies,²⁴⁻²⁶ but not all,²⁷ have shown a significant increase in the frequency of the e4 allele in the survivors of myocardial infarction or in the patients with angiographically verified coronary heart disease. Furthermore, the allele e4 has been observed to increase the risk of myocardial infarction at an early age.²⁸ These studies, however, have not included representative samples of non-diabetic population but highly selective groups of patients with established coronary heart disease. Differences in the prevalences of the e4 allele and coronary heart disease between populations also support the role of the e4 allele as a risk indicator for atherosclerosis. The Finns have exceptionally high prevalences of the e4 allele^{20,29} and coronary heart disease⁷ whereas in the Chinese³⁰ and Japanese³¹ populations the e4 allele is relatively uncommon, as is coronary heart disease.

ROLE OF E4 ALLELE

Why should people with non-insulin dependent diabetes be particularly susceptible to coronary heart disease if they happen to fall into the e4 allele category? Firstly, relatively high frequency of the apolipoprotein E phenotypes E4/4 and E4/3 in people with non-insulin dependent diabetes could lead to an increased risk of coronary heart disease. The distribution of the apolipoprotein E phenotypes in subjects with and without diabetes, however, was similar in our study and some other studies.³²⁻³⁴

Secondly, the risk for coronary heart disease could be related to the impact of the apolipoprotein E phenotypes on serum lipid and lipoprotein concentrations. Apolipoprotein E participates in the conversion of very low density lipoprotein remnants to low density lipoprotein, and this metabolic pathway is particularly effective in subjects with the e4 allele, which might result in a higher rate of production of low density lipoprotein in these subjects.^{1,35} Furthermore, a high density lipoprotein subfraction containing apolipoprotein E is considered to play an important part in the reverse cholesterol transport,³⁶ although the underlying mechanisms are poorly understood. Also subjects with the e4 allele have higher total and low density lipoprotein cholesterol concentrations,^{2,5} which increase the risk of coronary heart disease in the non-diabetic population.

High low density lipoprotein concentrations in people with the e4 allele seem to be related to the particularly effective absorption of cholesterol in these subjects,²⁹ which may be one explanation for the differences in low density lipoprotein concentrations among people with the various apolipoprotein E phenotypes. We found that total and low density lipoprotein cholesterol concentrations were higher in diabetic subjects with the e4 allele than in subjects with the e2 allele, confirming the findings of some previous studies.^{32,33} The low density lipoprotein cholesterol concentration of the subjects with phenotype E3/3 (62% of the study population) was similar to that in subjects with the e4 allele and higher than that in the

subjects with the e2 allele. But subjects with the E3/3 allele and non-insulin dependent diabetes still had a lower rate of coronary heart disease manifestations than those with the e4 allele.

No differences in other cardiovascular risk factors (smoking, hypertension, obesity, physical activity) were found between the different apolipoprotein E phenotypes (table IV), indicating that the susceptibility to coronary heart disease cannot be explained by these classic cardiovascular risk factors alone. Thus some additional mechanism related to the apolipoprotein E phenotype must be considered. Firstly, the modulation of lipoprotein metabolism by apolipoprotein E may not necessarily reflect the measured serum lipid and lipoprotein concentrations. Secondly, various mechanisms not related to the regulation of lipid and lipoprotein metabolism should also be considered. There is evidence that apolipoprotein E is involved in the immune system and tissue regeneration, which might indicate that it could also have a direct action on the atherogenic process at the cellular level in the arterial wall.³⁷⁻³⁹

CONCLUSIONS

Although the mechanisms for the association between coronary heart disease and the apolipoprotein E phenotypes in non-insulin dependent diabetes remain unexplained, our findings suggest that the apolipoprotein phenotypes E4/4 and E4/3 should be considered as important risk indicators for coronary heart disease in people with non-insulin dependent diabetes. The confirmation of apolipoprotein E polymorphism as an important contributory factor for coronary heart disease in non-insulin dependent diabetes warrants long term follow up studies based on representative population cohorts.

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